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ELECTRICAL ACTIVITIES OF THE ANTHOMEDUSAN, *SPIROCODON SALTATRIX* (TILESIUS)

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The functional organization of the nervous system in cnidarians has been studied mainly from the behavioral aspect (for review, see Bullock, 1965). The electrophysiological approach within the past decade has revealed a variety of types of pulses originating in nerves, muscles and non-nervous epithelia which function in a coordinated way for locomotory (Passano and McCullough, 1964; Mackie and Passano, 1968), feeding (Passano and McCullough, 1964, 1965; Mackie, 1968; Rushforth and Burke, 1971) and luminescent behavior (Morin and Cooke, 1971). The potentials recorded from hydrozoans typically show a long time course and are recordable over a wide area with little regional localization.

Some hydroids respond to light or shadow by reflex movements (Kikuchi, 1947; Passano, Mackie and Pavans de Ceccatty, 1967), by changing rhythmic activities (Passano and McCullough, 1962, 1964), by spawning (Yoshida, 1959). These responses are induced by dermal photoreception (Passano and McCullough, 1964) or mediated through the ocelli (Hisada, 1956; Passano, *et al.*, 1967), whose sensory cells conform electron microscopically with the photoreceptive structures of the ciliary type (Eakin and Westfall, 1962) and have neural connections with the outer nerve ring (Jha and Mackie, 1967; Mackie, 1971). Electrical activities associated with photic responses have been recorded in the dermal receptive system of *Hydra* (Passano and McCullough, 1964). Ocelli have been studied biochemically by techniques for pigment analysis (Yoshida, Ohtsuki and Suguri, 1967; Yoshida, 1969) but there have been no published investigations on the electrical activities of hydromedusae evoked by light-off.

The work reported below will deal with electrical activities, especially with those around the nerve ring of the hydromedusan *Spirocodon saltatrix,* a genus which is closely related to *Polyorchis.* As shown in Figure 1, the ocelli of *Spirocodon* are situated on the abaxial surface of each tentacular base and according to Uchida (1927), the first tentacles are formed in each perradius (Fig. 1A, center) and the next in each interradius (Fig. 1A, left and right ends). The number increases by the formation of subsequent tentacles on either side and slightly below those already formed. Thus, in fully grown medusa, the ocelli come to form an arch with the oldest one at its apex. At the same time the tissue of the radial streaks (Fig. 1, Rd S) extends, connecting the ocelli with the marginal ring structures. It is quite possible therefore that conduction pathways between the ocelli and the tentacles on the one hand and the nerve rings on the other are established across the intervening expanse of tentacular tissue (the subtentacular region), over a distance of 3–5 mm in most specimens, up to 8 mm in the largest one. This preparation is uniquely suitable for analysis of events occurring between the periphery (tentacles and ocelli) and the center (nerve ring).

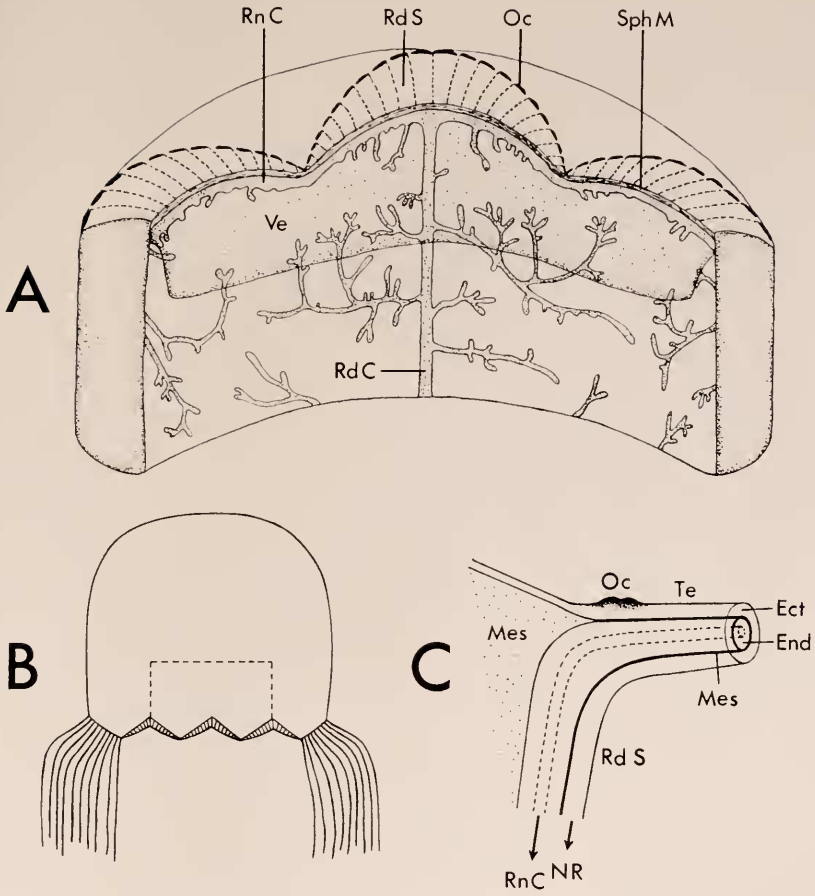

FIGURE 1. Semi-diagrammatic sketches of *Spirocodon saltatrix*. A is a part of the umbrella obtained by cutting along the broken line in B. In A, the velum is shown lying across the subumbrellar surface. The two nerve rings, the inner and the outer, which run circularly along the velar base, are omitted in order to avoid undue complexity. In B, the tentacles of the middle part are cut out. C shows a longitudinal section through the radial streak. Abbreviations used are: Ect, ectoderm; End, endoderm; Mes, mesogloea; NR, nerve ring; Oc, ocellus; Rd C, radial canal; Rd S, radial streak; Rn C, ring canal; Sph M, sphincter muscle; Te, tentacle; Ve, velum. For explanation, see text.

We have found various types of pulses, some possibly neural (narrower in pulse duration than any reported for hydroids) and some myonal, which occur both spontaneously and in response to electrical and photic stimulation. For reader's convenience, abbreviations and characteristics of the pulses recorded are tabulated in Table I.

METHODS

Specimens of the anthomedusa *Spirocodon saltatrix*, 5–7 cm in bell diameter, were collected from the Seto Inland Sea and kept in running sea water in a

TABLE I

Names, abbreviations and characteristics of pulses recorded from Spirocodon saltatrix

Name	Abbreviation	Figure	Characteristics				
			Pulse duration (msec)	Amplitude (mV)	Conduction velocity (cm/sec)	Possible source	Remarks
Marginal pulse	nMP	2A, B	5–10	0.3–1.5	71	Outer nerve ring	Coordinated with QSCP
Pre-swim pulse	PSP	3, dots	3–5	Less than 0.5	125	Outer and inner nerve ring	Trigger VSP and SSP indirectly
Quick and slow compound pulse	QSCP	4A, F	30–100	0.3–1	63*	Quick phase, possibly nerve	Coordinated with nMP
Slow monophasic pulse	SMP	4D, J	50–100	0.3–1	?	?	May conduct locally
Velar swim pulse	VSP	6A, B	250–500	1–4	?	Circular muscle of the velum	Consisted of two phases intervened by a steady phase
Subumbrellar swim pulse	SSP	6C, D	200–500	0.5–2	Circularly, 18 Radially, 6.6	Subumbrellar muscle	Consisted of two phases intervened by a steady phase
Epithelial pulse	EP	8A, B	15–30	0.4–1	12	Exumbrellar epithelium	—
Endodermal pulse	EDP	8D	200–400	1–5	?	Subumbrellar endoderm	Abolished by repetitive stimuli

* The figure was obtained only by one experiment.

laboratory tank. Figure 1A shows a portion of the umbrella which was removed from the area indicated by the broken line in Figure 1B. The tentacles were cut off distal to the ocelli, which are carried on the upper sides of the tentacles near their points of attachment to the exumbrella. Such a preparation contains on the subumbrellar side one radial canal (Rd C) at the center, a part of the ring canal (Rn C), sphincter muscle (Sph M) and velum (Ve), and on the exumbrellar surface one ocellar arch at the center with two halves of it on either side. The type of preparation was modified according to the experimental need and in some cases, smaller preparations having a half of the ocellar arch were employed.

The preparation was placed subumbrellar side up on a silver plate which served as the indifferent electrode and wetted from time to time by dropping sea water. A recording electrode was lowered vertically on to the preparation by means of micro-manipulator. For inspection, polarized light was admitted from below through a hole made in the center of the silver plate. The nerve ring is hard to see without staining, but its position can be estimated fairly accurately with the help of the strong birefringence of the adjacent sphincter muscle.

For photo-stimulation, light from a tungsten lamp (6V, 30W) was directed obliquely on to the preparation through a camera shutter.

Electrical stimuli were delivered through a pair of silver wires led from an isolator (Nihon Kohden, MSE-JM). Square pulses whose durations and amplitudes were in the range of 0.5–5 msec and 3–30V, respectively, were delivered to appropriate sites according to the experimental requirements.

Electrolytically polished stainless steel insect pins were used as recording electrodes. In some cases, the electrodes were connected to a coiled piece of 100 μ silver wire. Floating electrodes of this type were not dislodged by movements caused by vigorous muscle contractions.

Electrical response was recorded by series of electrical instruments manufactured by Nihon Kohden. Amplification was achieved by means of a direct coupled pre-amplifier (MZ-3B) and a main amplifier (AVH-2) with a long time constant. A condenser of 0.01 μF was connected in parallel with the input of the amplifier as an eraser of high frequency noise due to the pre-amplifier. The use of the condenser caused little change in the waveform of recorded potentials. For simultaneous recordings from two sites, another set of input box (AVB-JA) and a condenser coupled amplifier (AVB-2) with time constant of 0.3 sec were used. All the responses were displayed on a dual-beam cathode ray oscilloscope (VC-7A) and photographed by means of a continuous recording camera (PC-1B) from another oscilloscope (VC-MA-7).

Results

Pulses recorded from around the nerve ring

When the recording electrode was placed at points around the nerve ring, two types of quick pulse were recorded spontaneously or in response to electrical stimuli. One of them showed some of the characteristics of the events which Mackie and Passano (1968) termed "marginal pulses (MP's)" and for the reason to be described below we assumed that the pulses represent a predominantly nervous component of the MP's (nMP's). The other type of pulse was biphasic

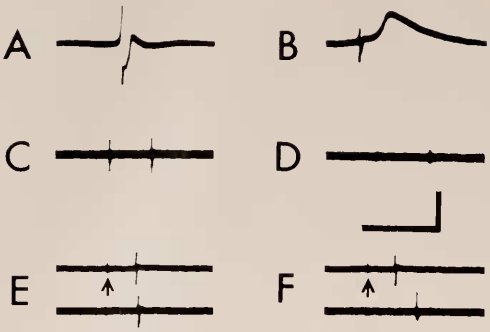

FIGURE 2. Pulses recorded from the outer nerve ring. A and B are nMP's shown by fast sweep speeds. Note a notch in A and a slow positive deflection after the nMP in B. C and D are records obtained from the isolated velum with the nerve ring. E and F are simultaneous recordings of nMP's evoked by electrical stimulation (arrows) on the outer nerve ring. Vertical bar shows 1 mV. Horizontal bar shows: 23 msec in A; 46 msec in B; 100 msec in C-F. Upward deflection is positive in this figure and also in the following figures.

events with amplitudes smaller than nMP's and they always preceded swimming contractions of the velum and the subumbrellar muscle. The term "pre-swim pulse (PSP)" therefore appears to be appropriate.

nMP's were generally triphasic with a small rebound and often had a notch on the recovering phase (Fig. 2A) and sometimes followed by a slow positive deflection (Fig. 2B). The waveforms of this type of pulse were quite variable and on rare occasions biphasic pulses or almost only a negative phase, were observed. Durations ranged from 5 to 10 msec. Amplitudes were usually 0.3 to 1.5 mV but became greatly reduced as the recording electrode was shifted away from the region immediately adjacent to the nerve ring towards the velum or the subumbrellar region. The same reduction in amplitude also occurred when the electrode was positioned on the inner nerve ring.

An attempt was made to isolate the nerve ring together with the velum from the margin of the umbrella, leaving the ring canal and the sphincter muscle on the bell side. Though clear-cut separation was practically impossible, large spontaneous pulses of the nMP type could be recorded along the base of the isolated velum, probably the outer nerve ring (Fig. 2C). The responses, however, became considerably smaller when the electrode was shifted away about 0.5 mm towards the velum (Fig. 2D). It appears likely, therefore, that nMP's are restricted to the velar edge, possibly to the outer nerve ring.

The nMP's appeared in sequence with a short time lag when they were recorded simultaneously at two sites on the nerve ring (Fig. 2E). When an incision was made through the nerve ring between the two electrodes using a fine razor tip, the coordination of events on the two sides of the cut either persisted or was only temporarily abolished. In either case, the time lag between the response peaks at the two points became markedly longer (Fig. 2F), indicating that coordination can be achieved by some kind of alternative route which exists in surrounding tissues. A further cut across the subtentacular region up to the level of the ocelli resulted in complete abolition of coordinated events even though the velum and the subumbrellar muscle sheet are almost intact. The alternative conduction pathway

must therefore lie in the subtentacular region and not in the subumbrellar or velar regions. However, since the nerve rings cannot be cut without damaging adjacent tissues to some extent, the possibility cannot be excluded that some conduction occurs through portions of the subumbrellar and velum lying immediately beside the nerve ring.

Conduction velocities at temperatures of 16–20° C were measured by estimating the delay between the response peaks recorded on the two electrodes following electrical stimulation on the nerve ring. In each preparation they were measured 3–7 times and the values were averaged. The mean value of averages obtained from 6 animals was 70.8 cm/sec (variation, 59.4–81.4 cm/sec).

PSP's were small biphasic events with amplitudes of less than 0.5 mV and durations of 3–5 msec (Fig. 3A). When recordings were done on the outer nerve ring, these pulses were always followed by a slow negative deflection (Fig. 3B). Additionally, the negative deflections were frequently followed by a complex pattern of pulses (Fig. 3C) associated with muscle contraction. Thus it appears that the small biphasic events trigger the swimming muscular movements indirectly through the slow negative deflection. Here, it must be noted that the waveform and the polarity of both the negative deflection and the muscle contraction pulse were greatly changed when recordings were made on the inner nerve ring (Fig. 3D). These points will be described later.

PSP's were not restricted to the outer nerve ring as was the case for nMP's but were recordable on the inner nerve ring without appreciable change in amplitude. However, shifting the electrode away from the nerve ring resulted in an abrupt decrease in amplitude, suggesting that PSP's were generated in the two nerve rings, the inner and the outer.

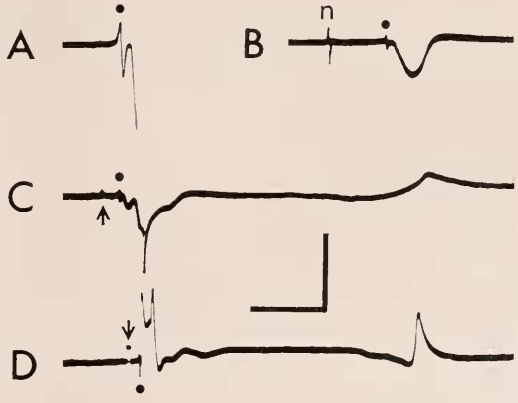

FIGURE 3. Pulses (PSP's) related to trigger muscle contractions. PSP's are indicated by dots and the nMP, by "n." A and B are PSP's shown by fast sweep speeds and C and D show contraction pulses after the PSP's. Note that in B the PSP is followed by a slow negative deflection. C is a record obtained on the outer nerve ring and D, on the inner nerve ring. Vertical bar shows: 0.8 mV in A and 2 mV in B-D. Horizontal bar shows: 23 msec in A; 50 msec in B; 100 msec in C and D. Artifacts by electrical stimulation are indicated by arrows.

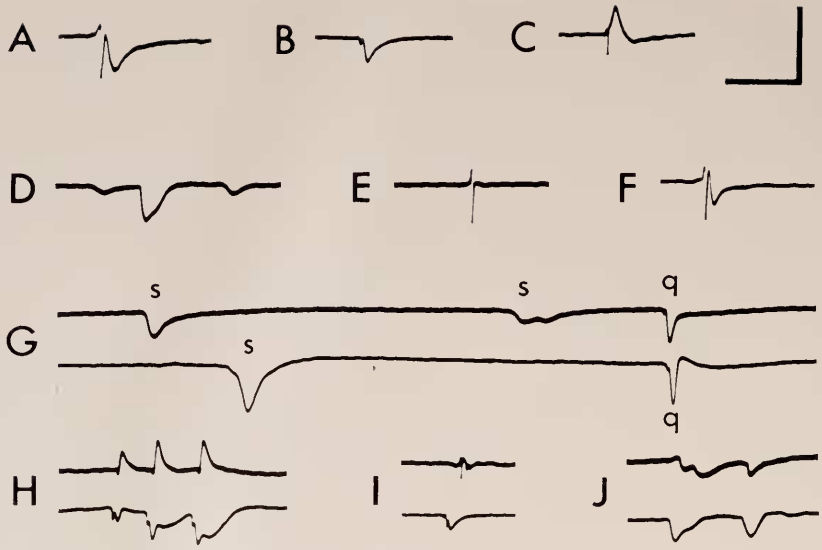

FIGURE 4. Pulses recorded from the subtentacular region and tentacles. A-E are records obtained from the subtentacular region and F, from the tentacle. A and F are records obtained by fast sweep speeds to show waveforms of QSCP's. G shows simultaneous recordings obtained from two radial streaks separated by several radial streaks (s, SMP's; q, QSCP's). In H, the upper trace is a record obtained from the tentacle and the lower trace, from the subtentacular region. I shows simultaneous recordings obtained from two tentacles separated by several radial streaks. J shows simultaneous recordings obtained at two sites along the same radial streak. Vertical bar shows: 2 mV in B, D, H and the upper traces of G and J; 0.8 mV in A, C, E, F, I and the lower trace of G; 1.6 mV in the lower trace of J. Horizontal bar shows: 46 msec in A and F; 100 msec in the others.

Conduction velocities along the nerve ring were measured under the same condition as nMP's. The mean value obtained from 5 animals was 125 cm/sec (variation, 115–142 cm/sec).

Pulses from the subtentacular region and tentacles

Two kinds of pulses were recorded in the subtentacular region. One of them was a compound event consisting of a quick pulse followed by a slower component usually negative (Fig. 4A, B) but occasionally became positive (C). The second type of pulse was a slow negative monophasic pulse (D). Both of these also occurred spontaneously on the nerve ring-free preparations. For convenience sake, the former will be called a quick and slow compound pulse (QSCP) and the latter, a slow monophasic pulse (SMP). In QSCP's, the ratio of the amplitudes of the quick and slow phases was variable, as in Figure 4A and B, and on rare occasions the slow phase was hardly detectable (E).

Since the tentacles are continuous with the radial streaks (Fig. 1C), pulses recorded from tentacles would be expected to show similarities to those recorded from the subtentacular region. Indeed, not only QSCP's (F) but also the SMP's were recordable from the tentacles.

QSCP's appear to be conductive events because simultaneous recordings at two sites in the subtentacular region showed the synchronous or nearly synchronous appearance (Fig. 4G, q). The same was true in preparations of the subtentacular region separated from the nerve ring. Similarly, coordinated QSCP's were obtained in recordings from a radial streak of one tentacle and the tentacle associated with another radial streak several millimeters away (Fig. 4H). Further, coordinated firings were also recorded from two tentacles separated by several radial streaks in the nerve ring-free preparation (Fig. 4I).

On the other hand, SMP's seem to be conducted for a short distance only. As shown in Figure 4G three SMP's (s) occurred independently when the recording sites were separated by several radial streaks. This tendency for independent firings was also detected between adjacent radial streaks. On the same radial streak, coordination, though weak, could be achieved with a slight time lag (Fig. 4J). Conduction velocities could not be measured owing to the short distance involved and the irregular pulse form. Thus SMP's appear to be conducted locally within each radial streak but not across the radial streaks.

The interrelationship between nMP's and QSCP's is important. Though the former usually preceded the latter (Fig. 5A), this does not mean that QSCP's are exclusively efferent events. Indeed as shown in Figure 5B, spontaneous QSCP's preceding nMP's were sometimes observed. The reciprocal elicitation of nMP's and QSCP's implies that the nerve ring, the subtentacular region and the tentacles form one through conducting system connected mutually all over the area and that the relay circuit whose existence is required to explain the continued coordination of nMP's following incision of the nerve rings between two recording sites (see above) is probably achieved by the QSCP's in the subtentacular region.

The non-polarized conduction of the QSCP's can be unequivocally demonstrated by recordings made from animals with intact tentacles. Two recording electrodes were placed about 1 cm apart on the same tentacle and electrical stimuli were delivered either to a place more distal to the recording sites on the same tentacle (Fig. 5C) or to a tentacle separated by 6 radial streaks from the recording sites (Fig. 5D). In Figure 5C, the sequence of events was from distal (lower record) to proximal (upper), whereas in Figure 5D, the sequence was reversed, *i.e.*, from

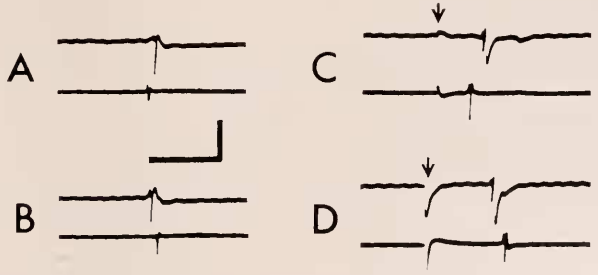

FIGURE 5. Non-polarized conduction of QSCP's and their relation to nMP's. In A and B, the upper traces show spontaneous pulses obtained from the subtentacular region showing QSCP's and the lower traces, from the outer nerve ring showing nMP's. C and D are records obtained by electrical stimulation (arrows) from the tentacles showing QSCP's. Vertical bar shows: 1 mV in C, D and the upper traces of A and B; 2 mV in the lower traces of A and B. Horizontal bar shows 100 msec.

proximal (upper) to distal (lower). The conduction of QSCP's must therefore be non-polarized. In both cases, the time lag between the two pulses was 16 msec so that a rough estimation of the conduction velocity of the QSCP's along the tentacle would be 63 cm/sec. It follows from these observations that excitation can travel up a tentacle proximally in the form of QSCP's, reach the radial streak, and then propagate circularly towards other tentacles via the subtentacular region and pass distally to the tentacle. The latencies after stimulus in Figure 5D being larger than in Figure 5C can be explained by the longer distance for the pulses to travel.

Contraction pulses of the velum and the subumbrellar muscle sheet

When the recording electrode was placed on the subumbrellar side of the velum, two successive slow potential changes separated by a steady phase were recorded (Fig. 6A, B). Their amplitudes ranged from 1–4 mV and the durations measured from the beginning of the first phase to the end of the second one were 250–500 msec. The coupled occurrence of the two potential changes was also observed on the subumbrellar muscle sheet (Fig. 6C, D; amplitudes, 0.5–2 mV;

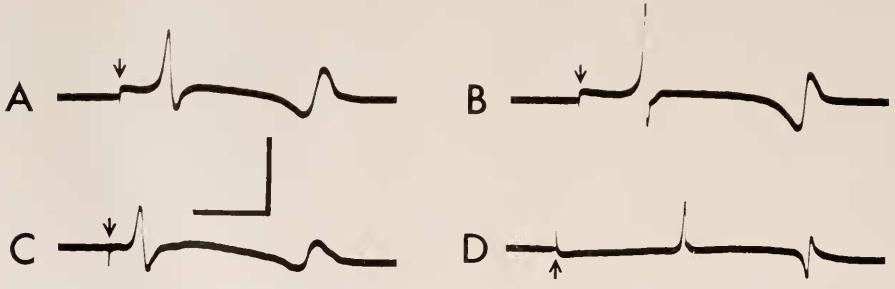

FIGURE 6. The contraction pulses of the velar and the subumbrellar muscles. A and B are records obtained on the subumbrellar-side surface of the velum and C and D, on the subumbrellar muscle sheet. Arrows indicate artifacts by electrical stimulation. All recordings were done with the floating electrode. Vertical bar shows 2 mV. Horizontal bar shows 100 msec.

durations, 200–500 msec) but here the waveforms were somewhat variable, the first phases sometimes taking a multiple form. The two types of pulses from the velum and the subumbrella resembled each other closely in waveform (Fig. 6, A to C and B to D). They always occurred in association with swimming movements of the respective muscle tissues. We therefore call them the velar swim pulse (VSP) and the subumbrellar swim pulse (SSP's). The complicated waveform following the PSP shown in Figure 3D probably represents a composite event of the two types of swim pulses.

It is interesting that conduction over the surface of the subumbrella was much faster circularly than radially. Conduction velocities of SSP's were estimated from time differences using the peaks of the first phases as recorded at two electrodes following stimulation. The values as obtained from 5 animals at 16–21° C were found to vary from 13.4–24.5 cm/sec (18.2 cm/sec on average) for cir-

cular conduction and from 4.8–8.8 cm/sec (6.6 cm/sec on average) for radial conduction.

The polarity of the first phase depends on the site of the active electrode with respect to the indifferent one (Fig. 7). Here, the velum was dissected away from the nerve ring and the active electrode was placed either on the subumbrellar (A, left) or the exumbrellar (A, right) side of the same isolated velum, placed on the indifferent electrode. The first phase always appeared in opposite polarities in such recordings while the polarity of the second phase was usually unaltered.

Electrical activities over the subumbrellar surface were studied in more detail. According to Mackie and Passano (1968) the umbrellas of *Euphysa* and *Sarsia* consist of 5 layers, *i.e.*, the subumbrellar ectoderm, mesogloea, endodermal lamella, again mesogloea and exumbrellar ectoderm. In *Spirocodon*, the umbrellar tissues could also be separated into five layers under the polarized dissecting microscope. The first, consisting of thin regular and fibrous components, could be torn off by

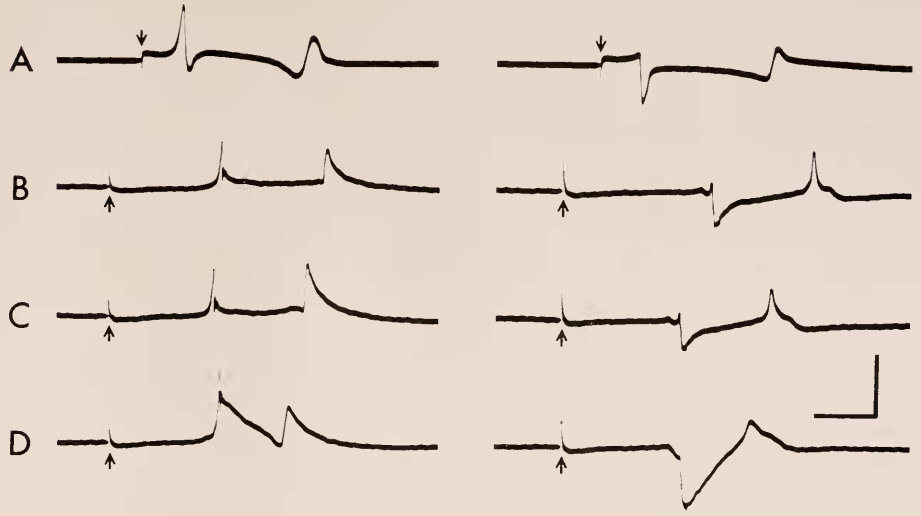

FIGURE 7. Polarity reversal of VSP's and SSP's. A shows VSP's recorded on the subumbrellar side (left) and on the exumbrellar side (right) of the velum. B-D show SSP's obtained by successive electrical stimuli from the subumbrellar muscle sheet (left) and from the mesogloeal side (right). Stimulus artifacts are shown by arrows. Vertical bar shows: 2 mV in A and 0.8 mV in B-D. Horizontal bar shows 100 msec.

means of a fine forceps in the circular direction, which is the direction in which the muscle is orientated. This layer is probably the subumbrellar ectoderm. The second, probably the mesogloea, was also thin but not directional. Beneath it there was a slightly opaque and very thin layer, presumably the endoderm. The presumed endoderm could not be exposed uninjured. On peeling this layer off, the transparent and thick mesogloea again appeared. The mesogloea was covered by the exumbrellar ectoderm which was vulnerable to and difficult to isolate.

In the following experiments, a part of the subumbrellar muscle sheet and the thin mesogloea supporting it were isolated from the umbrella by means of a fine

razor. The responses in Figure 7B, C and D were evoked by three successive stimuli (B to D) delivered at intervals of 0.9–1.3 sec. The left column shows records with the subumbrellar muscle side up, the mesogloea side being applied to the indifferent electrode. The SSP's showed waveforms similar to those of intact tissues. The increased positivity of the first phase both in amplitude and in the falling phase which appeared in response to the 3rd stimulus (D, left) was often observed after several repetitive stimuli. The recordings of the right column were done after the same preparation was turned over, and the electode was repositioned in the site corresponding to that in the previous recordings. It is noteworthy that the first phase, being positive on the left, became negative on the right and that in D, the increased positivity of the first phase on the left reflected as the increase in negativity on the right. On the other hand, the polarity of the second phase, being only positive in this case, did not alter in spite of the changes in the waveforms.

Pulses on the exumbrellar ectoderm and the endodermal lamella

It has been described in some hydromedusae that the exumbrellar ectoderm and the subumbrellar endoderm are completely nerve free, but that conductive pulses are recordable from them (Mackie and Passano, 1968). Figure 8A and B shows pulses evoked on the exumbrella by electrical stimuli. They were usually diphasic with amplitudes of 0.4–1.0 mV (A), and sometimes showed a notch in the course of the deflection (B), suggesting the existence of multiple components. Conduction velocities were measured at 16–21° C, using 6 animals and 2 measure-

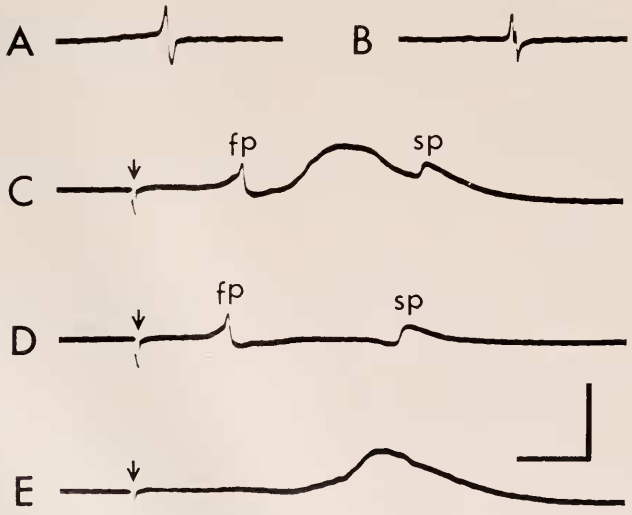

FIGURE 8. Epithelial pulses (EP's) and endodermal pulses (EDP's). A and B show EP's recorded on the exumbrella. C and D are records obtained by successive stimuli on the intact subumbrellar muscle sheet, showing SSP's whose first and second phases are indicated by fp's and sp's. The EDP in between the two phases in C disappeared in D. E shows the EDP recorded on the mesogloea after the subumbrellar muscle sheet was peeled off. Vertical bar shows: 0.8 mV in A and B; 2 mV in C-E. Horizontal bar shows 100 msec.

ments on each. The mean value was 12.2 cm/sec (variation, 7.8–14.3 cm/sec). Their waveforms were like those of nMP's to some extent but it was easy to distinguish them from nMP's by virtue of the much longer durations (15–30 msec) and the slower conduction velocities. They are possibly the epithelial pulses widely observed in hydromedusae and will be abbreviated as "EP". The relation between the EP's and all the other pulses still remains uncertain.

Slow deflections, monophasic or with multiple peaks, could be recorded on the subumbrellar muscle sheet. They were often accompanied by SSP's (Fig. 8C), but when repetitive stimuli at 1 cycle/sec were delivered to the subumbrella, this new type of pulses disappeared after one or two responses, leaving only the SSP's (Fig. 8D). On the other hand, removal of the subumbrellar muscle sheet resulted in complete abolition of the SSP as expected but the slow monophasic deflections still remained (Fig. 8E). Stripping off the mesogloea on the subumbrellar side, which was destructive for the endodermal cells, caused disappearance of this pulse. It follows then that the present type of pulse is not originated in the subumbrellar muscle but in the endodermal lamella. It will be called an endodermal pulse (EDP).

Electrical activities induced by shading

As described above, *Spirocodon* responds to shading by initiating swimming pulsations. The shadow reflex must involve a central coordinating pathway such as the nerve ring. Indeed, in response to light-off the pulses except for EP's and EDP's appeared singly or in combination. An example is shown in Figure 9A in which the PSP, the VSP and the nMP appeared in sequence on the outer nerve ring. The latencies measured from the light-off to the first pulse ranged between 0.2 and 1.1 sec irrespective of the pulse types. Responses were never observed at "on" of a strong light after a period of dark adaptation, however long.

When a cut was made in parallel with a line of the ocelli across the radial streaks, responses were completely abolished on the other side of the ocelli. On the ocellar side, however, both QSCP's and SMP's were induced in response to light-off (Fig. 9B). Further, as shown in Figure 9C the QSCP recorded on a tentacle was undoubtedly conducted from the proximal side (lower trace) to the distal end (upper trace). It appears as if the QSCP was information carrier of light-off generated in the ocelli. However, though QSCP's and SMP's are evoked by light-off without the nerve ring, they cannot be assumed to be the information carrier because upon shading QSCP's usually appeared after nMP's and appearance of SMP's does not coordinate with pulses in the nerve ring (Fig. 9D, E). There must be an event or events which carry information of shading from ocelli through the subtentacular region towards the nerve ring and trigger nMP's, PSP's and indirectly muscle contraction pulses (SSP's, VSP's). At present, we have not observed such an electrical correlate to carry information of shading.

Discussion

Nervous activities in hydrozoa have been studied mainly from the behavioral point of view (for review, see Bullock, 1965) and it is ironic that an important outcome of the recent work is the finding of non-nervous, or neuroid conduction.

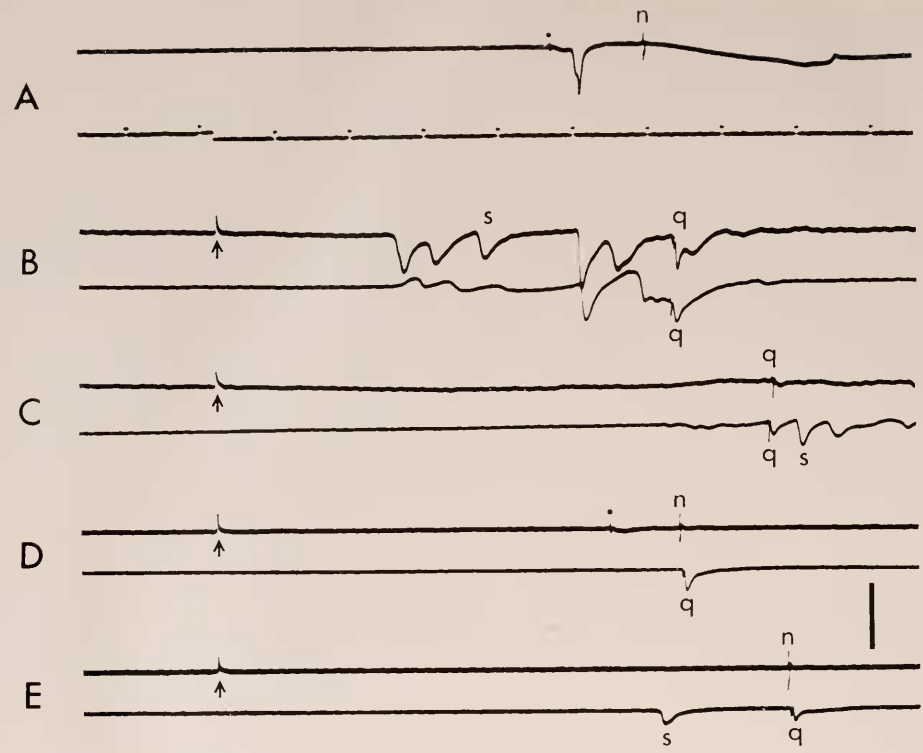

FIGURE 9. Light-off responses of various types of pulses. The upper trace of A is a record from the outer nerve ring. In B, the upper trace is a record from the tentacular base and the lower trace, from the subtentacular region. In C, the upper trace is a record from the distal side of the tentacle and lower trace, from the proximal side of the same tentacle. Experiments in B and C were done on nerve ring-free preparations. In D and E, the upper traces are records on the outer nerve ring and the lower ones, from the subtentacular region. The downward shift of the lower trace of A shows light-off. Arrows indicate stimulus artifacts by light-off. The time mark in the lower trace of A shows 100 msec. B-E were swept at the same rate as A. PSP's are indicated by dots, nMP's by "n," QSCP's by "q" and SMP's by "s." Vertical bar shows: 2 mV in A, D and E; 0.8 mV in B and C.

In his review, Mackie (1970) is of opinion that pacemaker function is restricted to the nervous system in hydromedusae. Since Romanes (1876), rhythmic swimming pulsations in hydromedusae have been known to be under the control of pacemakers around the bell margin, but direct evidence of nerve impulses is lacking.

As mentioned above, we have recorded in *Spirocodon* two types of pulses (nMP's and PSP's) from around the nerve ring. One should be very cautious in assuming pulses taken from hydrozoans to be nervous in origin, for there is ample evidence that non-nervous epithelial pulses do occur (Mackie, 1965; Mackie and Passano, 1968; Josephson and Macklin, 1967). They are characterized by their long time course, slow conduction velocity and extensive distribution, while nervous events seem to be recorded only in the region of the nerve ring. Considering the restricted occurrence to the nerve ring and also the shorter duration and

the faster conduction velocity, nMP's and PSP's are best interpreted as reflections of nervous activities. The quick conduction of those pulses would be understandable if giant fibers, as demonstrated in the inner and the outer nerve rings of *Sarsia* (Mackie, 1971), are also present in *Spirocodon*.

As mentioned earlier, nMP's are considered to originate in the outer nerve ring, because of the reduction in amplitude when they are recorded from the inner nerve ring. The PSP's, on the other hand, trigger muscle activities (VSP's and SSP's), suggesting that they reflect activities of motoneurones. If the inner nerve ring consists, as suggested by Bullock (1965), mainly of motoneurones and interneurones, PSP's may be assumed to originate in the inner ring. Indeed, in contrast to nMP's, PSP's can be recorded without reduction in size on the inner nerve ring. The fact that PSP's occur on the outer nerve ring might be explained by reference to the observation that in several hydromedusae nerve fibers run from the inner nerve ring into the outer one across the mesogloea.

The durations of those pulses which have hitherto been assumed to be of nervous origin in hydrozoans are longer than 10 msec. In this respect, PSP's (3–5) msec) and nMP's (5–10 msec) are unusually short but not out of the range of those obtained from other classes of Cnidaria. Indeed, the pulse duration of the single nerve fiber of *Aurelia aurita* has been reported to be less than 0.6 msec (Bergström, 1971). However, it appears unlikely that nMP's picked up by metal electrodes reflect activities of single nerve fibers, because it is difficult to conceive that in the nerve ring which consists of a large number of nerve fibers, only one is active. Instead, we assume that the pulses picked up are mass potentials.

QSCP's are composed of two phases, the first quick one seemingly triggering the second slow one in its passage. The quick phase not only follows upon but also precedes nMP's. The conduction velocities of the two are roughly the same and therefore it may be inferred that the first phase is of nervous origin. The extensive distribution of the QSCP's as well as the direction of conduction, which can be either efferent or afferent, is not surprising, since diffuse nerve nets are widely known to occur within various epithelia in hydromedusae (Mackie and Passano, 1968). This type of pulse is most likely responsible for transmitting information between the periphery and the center, or between two remote sites in periphery. In this respect, it is rather difficult to understand why the QSCP's cannot play an afferent role for shadow signals.

It is significant that the polarity of the first phase of VSP's becomes reversed by turning over the preparation. This observation can be well explained by adopting the idea of an electrogenic layer which was first proposed for interpreting the polarity reversal of ERG by Tomita (1950). The electrogenic layer must lie in parallel with the surface of the velum, the subumbrellar side becoming more positive with respect to the exumbrellar side when excited. The recording from the subumbrellar side of the velum with the indifferent electrode along the exumbrellar side will result in the positive first phase and when reversed, the negative. When myoepithelial cells are excited a current flow might occur from the epithelial surface to the muscular bases of the myoepithelial cells, or alternatively a steady current which flows towards the epithelial surface in the resting state might decrease. What might make such a current flow is not clear, but it may be suggested that the structural inhomogeneity of myoepithelial cells could help to develop

a sink and source relationship in the cross-sectional direction. On the other hand, it is considered that the second phase whose polarity remains unaltered is evoked in a way common to the usual action potentials. The bundles of basal muscular processes might be responsible for generating the second component. The above arguments would also apply in the case of SSP's.

Spirocodon responded to light-off by firing various types of pulses and from the experiments shown in Figure 9, it is considered that the information of light-off originates first in the ocelli and is conducted to the nerve ring. Though the pulses which carry the original signal from the ocelli have not been recorded as yet, many pulses recorded in response to light-off give a clue to study information pathways from the photoreceptive sites to the center and hence to effectors such as the tentacles and locomotory apparatus.

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Summary

1. Electrical activities occurring spontaneously and in response to electrical or photic stimulation were recorded from nervous, muscular and non-nervous tissues of *Spirocodon saltatrix*.

2. Two types of quick pulses, nMP's and PSP's, were recorded from the nerve ring. nMP's originated in the outer nerve ring and PSP's, in both the outer and the inner nerve rings. The PSP's appeared to trigger swimming contractions because they always preceded the muscle contraction pulses of the bell (SSP's) and the velum (VSP's).

3. Composite pulses with quick and slow phases (QSCP's) and slow monophasic pulses (SMP's) were recorded on the subtentacular region and the tentacles, respectively. The QSCP's had an intimate relationship with nMP's and their quick components appeared to be of nervous origin as were nMP's and PSP's. The SMP's seemed to be myonal or epithelial events and their conduction was restricted to each radial streak.

4. Contraction pulses of the velum (VSP's) and the subumbrellar muscle (SSP's) consisted of two phases and a steady phase between the two. The electrogenic site of the first phase was discussed from the results of polarity reversal when the positions of the recording and the indifferent electrodes were reversed.

5. Pulses (EDP's and EP's) also occurred in response to electrical stimulation on the exumbrellar surface and the subumbrellar endodermal lamella, respectively.

6. All types of pulses except the EDP's and EP's occurred upon light-off but none of them responded to light-on. Though the information of light-off was considered to originate in each ocellus, no electrical correlate as regards the information carrier has been observed.

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